on gene expression. Interestingly, chromatin immunoprecipitation revealed Oct4, Nanog and Tcf3 co-occupy promoters of commonly regulated genes. The underlying mechanism by which Tcf3 is able to limit expression of active genes is being examined. Mutational analysis of TCF3 showed that the context-dependent regulatory domain (CRD) is required for the repression, suggesting that protein-protein interactions with the CRD are mediating TCF3 molecular activity in ESCs. Transfection experiments using Groucho proteins showed that TCF3 repression is Groucho-independent, since it is not affected by expression of a dominant negative GRG5 protein. To identify novel binding partners to TCF3-CRD, we have used a recombinant GST-TCF3-CRD protein as a bait to probe for interacting proteins in ESC nuclear extracts. The identity and functional significance of interacting proteins will help elucidate the molecular mechanisms involved in controlling the core regulatory circuitry controlling pluripotency.

doi:10.1016/j.ydbio.2008.05.370

Program/Abstract # 348
Wrestling with melanocyte development

Pei-Chih Lee, Kimberly M. Taylor, Carole LaBonne
Department of Biochemistry, Molecular Biology, and Cellular Biology, Northwestern University, IL, USA

Melanocytes in skin, hair and the choroid layer of the eye are derived from neural crest cells, a population of stem cell-like cells that are found only in vertebrate embryos. Microphthalmia-associated transcription factor (Mitf) and SoxE factors act as the key regulators during melanocyte development. Mis-regulation of Mitf or Sox10 can cause congenital diseases such as Waardenburg syndrome, as well as a dangerous skin cancer, melanoma. However, it is still unclear how these factors are regulated to control downstream target genes during melanocyte development. By co-expression of Mitf and SoxE factor, we demonstrated that these two factors synergistically activate the expression of melanogenic marker Dct, and the synergistic effect is modulated by the SUMOylation of either one of these proteins. This effect is achieved by SUMO-dependent alteration of transcriptional co-regulatory complexes. We provide evidence that SoxE, which previously had been described as a dedicated transcriptional activator, is actually a context-dependent transcriptional regulator that recruits a co-repressor when SUMOylated. We show that neither SoxE nor SUMO alone is sufficient to interact with this co-repressor, but rather recruitment requires bivalent interactions with both factors. These results and further studies on the regulation and cooperation of Mitf and SoxE factors can provide us a better understanding toward both normal melanocyte development and the melanocyte-related diseases.

doi:10.1016/j.ydbio.2008.05.371

Program/Abstract # 349
Transcriptional regulation of the FoxO1 gene during mouse development

Barbara Villarejo Balcells, Peter W.J. Rigby, Jaime J. Carvajal
The Institute of Cancer Research, Section of Gene Function and Regulation, Chester Beatty Laboratories, London, SW3 6JB, England, UK

FoxO1, a member of the Forkhead Box family of transcription factors, regulates many processes including cell cycle progression, differentiation and apoptosis. FoxO1 KO mice die early in gestation due to vascular defects. Comparative sequence analysis of >250 kb spanning FoxO1 reveals many non-coding sequences highly conserved between species that are likely to contain transcriptional control elements. We have used three overlapping BACs spanning the FoxO1 locus to define regulatory regions responsible for different subsets of expression. Using recombineering we have inserted a LacZ reporter gene at the translational start site in each BAC and generated transgenic mice.All BACs are able to drive LacZ expression in the umbilical cord, myotome and developing gut at various developmental stages suggesting sequences within the common interval (−38 kb to +104 kb) control expression in these regions. In addition, BAC38 and BAC61 show expression in the heart. Between 9.0 dpc and 11.0 dpc, BAC38 drives vascular expression that rapidly disappears by 11.5 dpc. In contrast, BAC61 only drives adult vascular expression suggesting two distinct vascular enhancers, an embryonic element between +104 kb and +148 kb, and an adult element between −61 kb and +104 kb. Finally, BAC116 is able to drive expression in the neural tube and cartilage indicating the presence of specific elements between −116 kb and −61 kb.In conclusion, FoxO1 expression is regulated by elements distributed over >264 kb acting at different times and anatomical locations.

doi:10.1016/j.ydbio.2008.05.372

Program/Abstract # 350
***Foxn1* is a regulatory target of Hoxc13 in ectodermal development and dysplasia**

Christopher S. Potter [a], Nathanael D. Pruett [a], Michael J. Kern [b], Alan R. Godwin [c], John P. Sundberg [d], Alexander Awgulewitsch [a]
[a] *Department of Medicine, Medical University of South Carolina, Charleston, SC, USA*
[b] *Department of Cell Biology and Anatomy, Medical University of South Carolina, Charleston, SC, USA*
[c] *Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, USA*
[d] *The Jackson Laboratory, Bar Harbor, ME, USA.*

Hoxc13 null (*Hoxc13*^{tm1Mrc}) mice share multiple phenotypic characteristics with the nude (*Foxn1*^{nu}) mouse, including hairlessness and a severe nail dystrophy. Previous DNA microarray and *in situ* hybridization data obtained with *Hoxc13* overexpressing mice indicated downregulation of *Foxn1* in the abnormally differentiated hair follicles of these mice. Considering the overlap of *Foxn1* and *Hoxc13* expression domains in the precortical region of anagen hair follicles and the presence of multiple *bona fide* Hoxc13 binding sites in the *Foxn1* promoter region, we hypothesize that *Foxn1* is a direct target of Hoxc13 regulation. Both genes are likely part of a regulatory network essential for both normal hair and nail plate development. We present a comparative analysis of *Hoxc13* overexpressing mice, *Hoxc13* null mice, and *Foxn1* null mice. Data obtained through immunohistochemistry, *in-situ* hybridization, real-time PCR, and Hoxc13-chromatin immuno-precipitation (ChIP) assays of the *Foxn1* promoter region, suggest that *Foxn1* is indeed a direct regulatory target of Hoxc13. Additionally, the evidence provided here suggests that *Hoxc13* is a key regulator of both normal hair and nail plate development and likely plays a central role in the complex regulatory networks underlying various forms of Ectodermal Dysplasia.

doi:10.1016/j.ydbio.2008.05.373